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1. Lechmann et al. (Sminars in Liver disease, 2000, Vol. 2, pp. 211-226).
2. Lechner et al. (Philos. Trans. R. Soc. Lond. B. Bio Sci. 2000, Vol. 355, pp. 1085-1092).
3. Purcel (Hepatology 1997, Vol. 26, pp. 11S-14S).

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The Hepatitis C Virus: Overview

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Our knowledge of hepatitis C virus (HCV) dates only from 1975, when non-A, non-B hepatitis was first recognized. It was not until 1989 that the genome of the virus was first cloned and sequenced, and expressed viral antigens used to develop serological assays for screening and diagnosis. HCV is in a separate genus of the virus family *Flaviviridae*. It is a spherical enveloped virus of approximately 50 nm in diameter. Its genome is a single-stranded linear RNA molecule of positive sense and consists of a 5′ noncoding region, a single large open reading frame, and a 3′ noncoding region. The open reading frame encodes at least three structural and six nonstructural proteins. The genome is characterized by significant genetic heterogeneity, based on which HCV isolates can be classified into six major genotypes and more than 50 subtypes. Even individual isolates of HCV are genetically heterogeneous (quasispecies diversity). Genetic heterogeneity of HCV is greatest in the amino-terminal end of the second envelope protein (hypervariable region 1). This region may represent a neutralization epitope that is under selective pressure from the host's humoral immune response. Infection with HCV proceeds to chronicity in more than 80% of cases, and even recovery does not protect against subsequent re-exposure to the virus. The development of a broadly protective vaccine against HCV will therefore require a better understanding of the molecular biology and immune response to this virus. (HEPATOLOGY 1997;26(Suppl 1):11S-14S.)

Before 1975, only two hepatitis viruses were recognized: hepatitis B virus (serum hepatitis virus) and hepatitis A virus (infectious hepatitis virus). Diagnostic tests for hepatitis B were first developed in 1964 and for hepatitis A in 1973. Shortly after the diagnostic tests for hepatitis A were developed, they were applied to sera from patients with non-B hepatitis acquired after transfusion. None of the cases was found to have been caused by hepatitis A, and such cases were termed non-A, non-B hepatitis.[1] At that time, non-A, non-B hepatitis accounted for as many as two thirds of transfusion-associated hepatitis cases. The agent and disease of non-A, non-B hepatitis became the subject of intensive research. The disease was transmitted to chimpanzees in 1978 by several groups, thereby establishing that it was caused by a transmissible agent. The chimpanzee provided a means of characterizing the transmissible agent by classical virological methods. By determining the infectivity titer of standard pools of plasma containing the virus and subjecting aliquots to various treatments, it was established that non-A, non-B hepatitis virus contained essential lipids (and was therefore enveloped) and had a diameter of approximately 30 to 60 nm.[2,3] However, it was not until 1989 that the genome of the agent was cloned and characterized and diagnostic tests were first developed.[4,5] Non-A, non-B hepatitis virus was subsequently renamed hepatitis C virus (HCV). Because its biological and molecular characteristics were most closely related to viruses of the family *Flaviviridae*, it was subsequently classified in a separate genus in this family.[6] HCV also shares slight sequence identity with other members of this virus family, especially the pestiviruses.[7] The pestiviruses, flaviviruses, and HCV now comprise separate genera within the *Flaviviridae*. These are named *Pestivirus*, *Flavivirus*, and *Hepacivirus*, respectively.

NATURE OF THE VIRION

HCV is a spherical enveloped virus of approximately 50 nm in diameter.[8] Virus recovered during the acute phase of infection from the plasma of naturally infected patients and experimentally infected chimpanzees has a buoyant density of approximately 1.06 g/cm^3 in sucrose.[9] In contrast, HCV recovered from cell culture after replication *in vitro* has a buoyant density of 1.12 g/cm^3 in sucrose.[10] The lower density of the serum-derived virus has been ascribed to its association with serum low-density lipoproteins, which appear to be bound to the virus.[11] These lipoproteins may facilitate entry of HCV into hepatocytes by endocytosis. HCV recovered from chronically infected individuals has a buoyant density of approximately 1.17 g/cm^3 in sucrose.[9] Such a higher density virus has been shown to be associated with antibody bound to the virus in antigen-antibody complexes. The association of HCV with low-density lipoproteins on the one hand and with antibody on the other may be important clues to the pathogenesis and natural history of HCV infection.

NATURE OF THE VIRAL GENOME

The genome of HCV is a single-stranded linear RNA of positive sense.[12] It is unsegmented and is approximately 9.5 kb in size (Fig. 1). A 5′ noncoding (NC) region consists of approximately 340 nucleotides, forms a stem-loop structure, and contains an apparent internal ribosomal entry site (IRES).[13] The IRES-like activity of the 5′ NC region has been demonstrated *in vitro* but is assumed to function *in vivo*. However, the IRES of HCV is less active than the IRES of other viruses, such as picornaviruses.

Immediately downstream of the 5′ NC region is a single

Abbreviations: HCV, hepatitis C virus; HVR1, hypervariable region 1; NC, noncoding; IRES, internal ribosomal entry site; ORF, opening reading frame; HVR2, hypervariable region 2.

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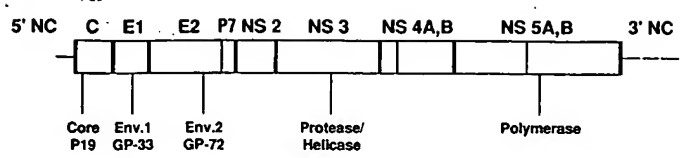

FIG. 1. The genomic organization of HCV. Abbreviations: C, core region; E, (env.) envelope region; NS, nonstructural region; P, protein.

large open reading frame of approximately 9,000 nucleotides, encoding a large polyprotein precursor of approximately 3,000 amino acids that is cotranslationally or posttranslationally cleaved into separate proteins by a combination of host and viral proteases. A capsid protein, at least two envelope proteins (E1 and E2), and a small putative protein of unknown function (P7) are encoded in the 5′ region of the open reading frame. At least six nonstructural proteins, including protease, helicase, and RNA polymerase enzymes and regulatory peptides are arrayed in the 3′ portion of the open reading frame. One regulatory protein, the product of gene 4a, is a multifunctional peptide that stabilizes and facilitates the proteinase activity of the amino-terminal quarter of the product of gene NS3 and, in addition, regulates the phosphorylation of the product of gene 5a.[14,15] The functions of the other putative regulatory proteins are poorly understood. Finally, there is a 3′ NC region that consists of approximately 50 nucleotides, a polypyrimidine (or polypurine) tract, and a recently discovered highly conserved terminal sequence of approximately 100 nucleotides.[16] Like the 5′ NC region, the 3′ NC region is thought to have considerable secondary structure.

GENETIC HETEROGENEITY: TYPES, SUBTYPES, AND QUASISPECIES

Perhaps the most important characteristic of the HCV genome is its sequence heterogeneity.[17] In this respect, it resembles the human immunodeficiency virus, a member of the virus family *Retroviridae*. The genetic heterogeneity of HCV is not uniform across the genome: the most highly conserved regions of the genome are parts of the 5′ NC region and the terminal 3′ NC region. The most highly conserved region of the open reading frame is the capsid gene. In contrast, the most heterogeneous portions of the genome are the genes encoding the envelope proteins. The 5′ end of the E2 gene is the most heterogeneous region of all and has been named the first hypervariable region (HVR1).[18,19] A few strains have a second HVR just 3′ of HVR1; HVR2 appears to be limited to strains of genotype 1b.[19] The HVR1 consists of approximately 90 nucleotides (30 amino acids) and is believed to be a major neutralization epitope of HCV.[20-23] Thus, its heterogeneity appears to be the result of selective pressures by the host's humoral immune system. The second heterogeneous region, HVR2, is only approximately 7 amino acids in length. Its significance is not well understood.

Based on their genetic heterogeneity, HCV strains can be divided into major groups, called types or genotypes. It has been proposed that genotypes be accorded species status. Within types, HCV isolates have been grouped into numerous subtypes. A total of six major genotypes has been identified and at least partially characterized. These have been designated types 1 through 6. Subtypes have been given letter designations. Thus, the most common HCV types are types 1a, 1b, 2a, and 2b. More than 50 subtypes have been described, and their nomenclature is confusing. Recently, strains identified principally in southeast Asia have been proposed as new major genotypes with designations of 7 through 11. However, more detailed phylogenetic analyses of such isolates strongly suggest that the isolates are actually members of genotypes 6 (proposed genotypes 7, 8, 9, and 11) or genotype 3 (proposed genotype 10).[24] The identification of these genetically diverse subtypes provides further evidence for the growing conviction that the so-called two-tiered classification system proposed by Simmonds et al[25] has been superseded by the realization that HCV heterogeneity may be a continuum. However, the concept of six major genotypes continues to be useful.

The major genotypes of HCV differ in their distributions worldwide. Genotypes 1, 2, and 3 and their subtypes are distributed worldwide. In contrast, genotype 4 appears to be a Pan-African type (the principal genotype in Zaire and Egypt), and genotype 5 has been found to be the principal genotype in South Africa. Genotype 6 and its many variants have been found principally in Asia. Based on an analysis of the genetic heterogeneity of HCV strains worldwide, it has been estimated that HCV genotypes diverged approximately 300 years ago.[26]

The clinical significance of the genetic heterogeneity of HCV remains elusive despite numerous studies of the possible effect of genotypes on pathogenesis and therapy. The results have been confusing, perhaps because the many confounding variables that are important in such an assessment have not always been taken into consideration, such as (in addition to genotype and subtype status) the quasispecies nature of individual isolates, including the number and divergence of variants present, sites of mutations, the titer of the virus, the clinical status of the patient, the duration of infection, and the local epidemiology, including age and social history (source and frequency of exposure, other confounding factors such as alcoholism, etc.). Many of these factors may be more important than genotype; some may be directly linked to genotype or not related except by epidemiology.

The quasispecies nature of HCV is emerging as a possible important predictor of the natural history of infection. A quasispecies is defined as a heterogeneous population of individual virions, each of which may be different at at least one genomic site. Usually a single dominant sequence is present that can change over time, being replaced by one or more minor populations as the result of external pressures on the quasispecies. One such pressure can be the immune system of the host. An estimate of the complexity of a quasispecies of HCV was determined by Farci et al.[23] They generated more than a hundred clones of the most genetically heterogeneous region of a single isolate of HCV (the H strain) and sequenced the clones to determine the dimensions of the quasispecies. They found almost 20 unique sequences (Fig. 2). The dominant sequence comprised two thirds of the clones, but at least 18 other sequences were also identified. Many of these were represented by a single clone (1% of the population). The genetic heterogeneity was confined largely to the HVR1 region of the viral genome.

IMMUNITY AND RESISTANCE TO INFECTION

Additional evidence that HVR1 is an important (perhaps neutralization) epitope of HCV comes from studies of this region in carriers of HCV. Carriers with an apparently normal

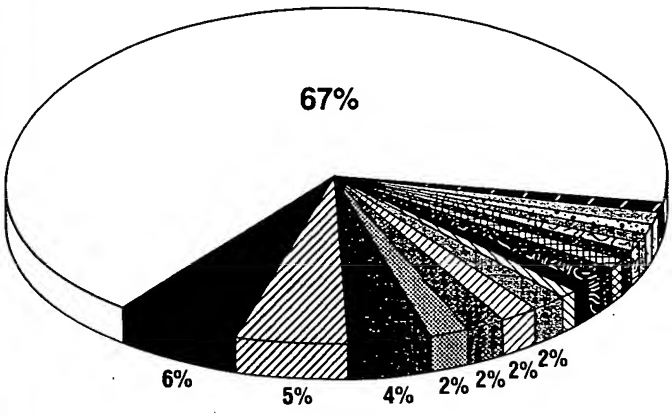

FIG. 2. Quasispecies of HCV, cloned from early acute phase plasma of patient "H" with hepatitis C. A total of 19 different sequences was identified among 104 clones that were sequenced. A single sequence was found in 67% of clones; other sequences represented minor clones that accounted for 1% to 6% of the total. Eleven clones were recovered as single copies. (Data from Farci et al.[23])

immune system display the normal degree of heterogeneity of HVR1; in contrast, patients with agammaglobulinemia who are HCV carriers demonstrate little or no genetic heterogeneity in HVR1[27] and patients chronically infected with HCV who are treated with interferon, a known stimulator of the immune response, display greater genetic heterogeneity of HVR1 than the other patient groups.[19,28]

The consequence of the genetic diversity of HCV is virus that has the ability to escape the immune surveillance of its host, leading to a high rate (>80%) of chronic infections and lack of immunity to re-infection in repeatedly exposed individuals. Both chronicity and lack of solid immunity probably result from the emergence of minor populations of the virus quasispecies that vary in sequence, especially in the HVR1. Data supporting this conclusion come from experimental infections of chimpanzees that developed repeated infections with HCV after up to 4 sequential inoculations with the virus.[29,30] Not only were the chimpanzees not protected after re-inoculation with a different virus isolate, they were also not protected from re-infection following re-inoculation with the same virus isolate. Similarly, children with thalassemia major undergoing repeated transfusions of blood in Sardinia were found to be re-infected and to experience a second case of clinical hepatitis C following re-exposure, even when the re-exposure was with a virus very closely related to the original infecting virus.[31] Thus, there was no evidence for lasting immunity after HCV infection.

Similar conclusions regarding lack of immunity to re-exposure can be drawn from attempts to vaccinate chimpanzees with recombinant HCV envelope antigens expressed in eukaryotic cells. Chimpanzees vaccinated with a heterodimer of recombinant HCV E1 and E2 proteins were, for the most part, protected against infection (in some cases) or chronic infection (in other cases) after intravenous challenge with 10 chimpanzee-infectious doses of the homologous virus. However, when chimpanzees that resisted infection after challenge with the homologous virus were re-vaccinated and challenged with 64 chimpanzee-infectious doses of a closely related HCV of the same genotype and subtype (1a), they were not protected.[32]

Attempts to correlate observations of HCV *in vivo* with measurement of neutralizing antibody *in vitro* have yielded confusing and controversial results. Neutralization experiments involving the inoculation of certain continuous lymphocyte lines that are susceptible to HCV infection and *in vitro* neutralization experiments involving inoculation of chimpanzees with the antigen-antibody mix have pointed to HVR1 as a major neutralization epitope of HCV.[20,21] However, neutralization-of-blocking assays based on antibody-mediated blocking of binding of recombinant HCV envelope proteins to cell lines thought to be susceptible to HCV replication have suggested that neutralization of the virus is mediated by one or more epitopes elsewhere in the envelope proteins.[33] If the HVR1 proves to be the most important neutralization epitope, it would probably be difficult to develop a broadly protective vaccine against HCV.

Despite this pessimism regarding the nature of immunity in HCV infection, there may be some reason for hope that HCV can be prevented by immunoprophylaxis. Double-blind placebo-controlled trials of normal immune globulin for the prevention of transfusion-associated non-A, non-B hepatitis (most of which was hepatitis C) conducted in the 1970s and 1980s revealed that significant protection could be achieved against symptomatic and icteric non-A, non-B hepatitis as well as chronic disease if globulin was administered before transfusion.[34-37] Similarly, when plasma units containing antibody to HCV were screened from pools of plasma destined for fractionation into blood products, the resultant lots of intravenous immune globulin were associated with a high incidence of hepatitis C in recipients, in contrast to results obtained with most lots of intravenous immune globulin prepared before antibody to HCV–positive plasma units were removed.[38,39] Both of these observations strongly suggest that pooled plasma contains a mixture of antibodies to HCV that is capable of neutralizing diverse HCV strains found in nature. Thus, the neutralization epitopes of HCV must be finite in their diversity. If the breadth of this diversity can be mapped, it may be possible to construct a polyvalent vaccine that can protect against most, if not all, HCV variants.

CONCLUSION

In summary, HCV remains a challenge both to the physician and the virologist. Gaps in our knowledge about this virus include the important question of the significance, if any, of genotypes and subtypes to pathogenesis and vaccine development. Needs for future research include the development of reproducible and useful cell culture systems that can be used for the development of reproducible and meaningful assays for neutralizing antibody to HCV. Future research must be directed toward the development of broadly protective vaccine and truly effective therapeutic agents. Achievement of these goals will likely require a better understanding of the molecular biology of HCV and the host's immune response to the virus.

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